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ENDOTHIA PIGMENTS II

ENDOTHINE RED[1]

CHAS. E. SANDO

In 1911 Panatanelli[2] noted a yellow pigment occurring in *Endothia parasitica* and attempted to identify the substance as a lipochrome. Later, Anderson[3] claimed that the pigment was closely related to aurin. Shear and Stevens[4] were the first to point out that certain species of Endothia produced a "perilla purple" color when grown on starchy media. In a general study of the pigments produced by species of Endothia, the results of which are presented in the first paper of this series, Hawkins and Stevens[5] showed that three distinct coloring matters are elaborated. Two of these are yellow and have been designated pigments A and C, and the other, which is a brilliant red, has been designated pigment B. With regard to the similarity of any of these pigments to a lipochrome, these authors make the following statement:

"It is obvious then that these pigments are lacking in many of the properties of lipochrome and there is little reason at present for assuming that they belong in this rather indefinite group."

They do state, however, that the pigments from *E. parasitica* bear some similarity to aurin, but not enough to warrant the conclusion that they are the same.

All three pigments described by Hawkins and Stevens are found in *Endothia fluens*, pigment B abundantly. This species therefore afforded an excellent source of material for a further chemical study of the red pigment. This paper deals entirely with the chemistry of the coloring matter designated as pigment B by Hawkins and Stevens, which is probably the source of the "perilla purple" of Shear and Stevens. Since the substance appears to be a new coloring matter, the name *endothine red* is proposed.

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[1] The work here reported was carried out under the general supervision of Dr. Lon A. Hawkins as a part of a cooperation between the Office of Plant Physiological and Fermentation Investigations and the Office of Horticultural and Pomological Investigations, Bureau of Plant Industry. Published by permission of the Secretary of Agriclture.

[2] Panatanelli, E. Sul parassitismo di *Diaporthe parasitica* Murr. per il Castagno. Rendiconti della Accad. Lincei Roma, Classe di Sceinze, Fisiche, Matematiche e Naturali. V. **20**: 366–372. 1911.

[3] Anderson, P. J. The morphology and life history of the chestnut-blight fungus. Bull. Penn. Chestnut Tree Blight Comm. **7**: 1–43. 1913.

[4] Shear, C. L., and Stevens, Neil E. Cultural characters of the chestnut-blight fungus and its near relatives. U. S. Dept. Agr. Bur. Pl. Ind. Circ. **131**: 3–18. 1913.

[5] Hawkins, Lon A., and Stevens, Neil E. Endothia pigments. I. Amer. Journ. Bot. **4**: 336–353. 1917.

EXPERIMENTAL

For the purpose of obtaining a sufficient quantity[1] of pigment for investigation, *Endothia fluens* was grown in flasks on sterilized rice. The culture medium with the mycelium was dried and ground, and the coloring matter obtained in the manner described by Hawkins and Stevens. The method is briefly as follows:

A cold alcoholic extract of the powder was evaporated under vacuum until most of the alcohol had been removed, and the residue, which consisted of a mixture of the impure yellow and red substances, was treated with a large volume of water. This treatment caused a heavy reddish-yellow precipitate to separate, leaving the supernatant liquid dark red. A smaller amount of the red compound was recovered from the mother liquor. The reddish-yellow precipitate after thorough extraction with ether left a residue which consisted mostly of endothine red in a semi-crystalline state. By repeated recrystallization from alcohol it was finally obtained comparatively pure. It was possible, also, to purify the pigment by dissolving it in boiling water and allowing it to stand for several days.

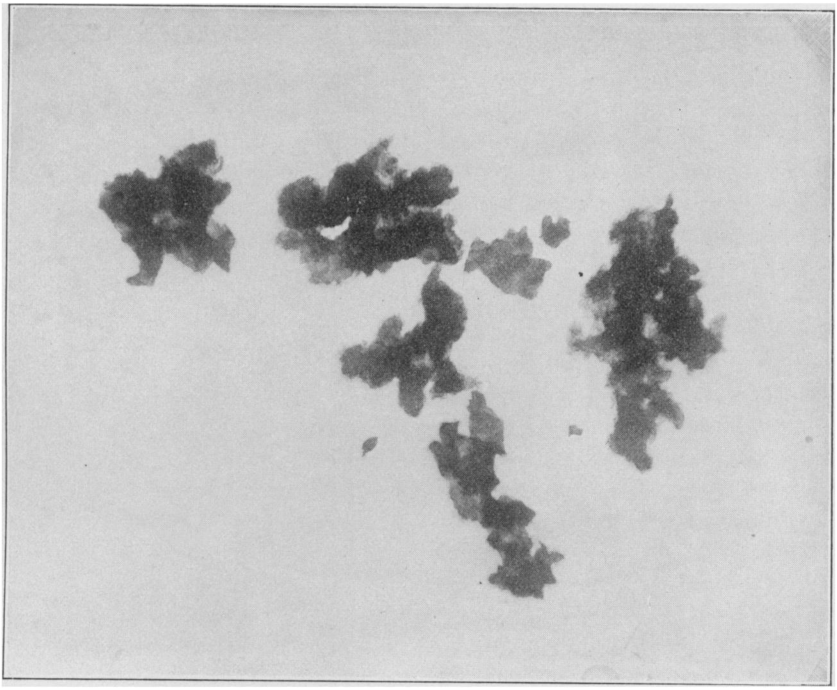

FIG. 1. Photomicrograph of crystal aggregates of endothine red deposited from water.

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[6] The author is indebted to Dr. Hawkins for most of the crude material used in this investigation.

The coloring matter was deposited from water as a mass of crystalline aggregates, as illustrated in figure 1.

The substance, crystallized from alcohol (figure 2), forms glittering "ferruginous"[1] thin plates having a metallic luster. It is readily soluble in pyridine, slightly soluble in cold ethyl alcohol, methyl alcohol, acetic ether, ether, and acetone; very sparingly soluble in cold water, and insoluble in acetic acid, toluene, benzene, carbon tetrachloride, chloroform, carbon disulphide, and petroleum ether. By warming, it is readily soluble in alcohol, but sparingly in water, acetic ether, and acetone. When warmed with glycerine on a water bath a red solution results.

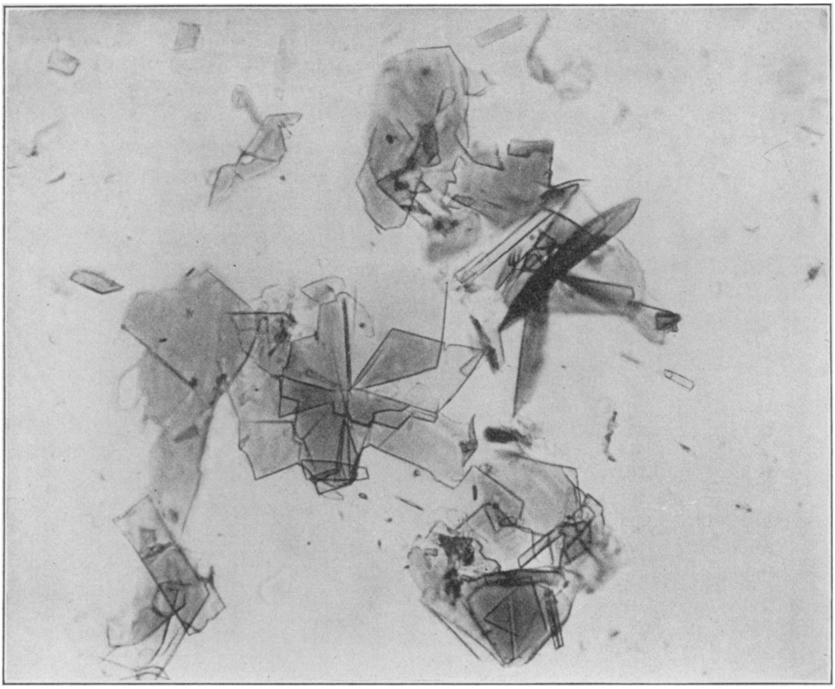

FIG. 2. Photomicrograph of crystals of endothine red purified from alcohol.

Dilute alkalis dissolve it with a violet red color; the addition of dilute acid to this solution changes it to yellow.

Concentrated sulphuric acid gives a "perilla purple" solution.

Cold concentrated nitric acid has no effect on it even after standing 24 hours, but upon gentle warming a faintly colored solution results.

Alcoholic ferric chloride produces with very dilute solution of the pigment a greenish "raw sienna" color; with more concentrated solutions a "dull greenish-black" precipitate is obtained.

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[7] Color descriptions placed in quotations indicate that they were taken from Ridgway, Robert, Color standards and nomenclature. Washington, D. C., 1912.

Alcoholic solutions of sodium and barium acetates yield insoluble sodium and barium salts.

Silver nitrate and Fehling's solution are both reduced, the former even in the cold.

When heated between watch glasses a portion of the substance sublimes without melting, giving rise to a "Brazil red" sublimate. A carbonaceous mass remains in the lower watch crystal.

Combustions were made of separate portions of the pigment, one purified from alcohol, dried at 150°–200° C., and the other purified from water and dried at 100° C. The results are given in table 1.

TABLE 1. *Combustions of Endothine Red*

Samples *A* and *B* from alcohol and samples *C* and *D* from water.

Sample	Weight	CO_2	H_2O	C, Percent	H, Percent	O, Percent
A	.1719	.3467	.0543	55.00	3.53	41.47
B	.1591	.3186	.0509	54.62	3.58	41.80
C	.1572	.3136	.0504	54.40	3.58	42.02
D	.1803	.3628	.0565	54.87	3.51	41.62
Arithmetic mean of four determinations				54.73	3.55	41.72
Required for $C_7H_5O_4$				54.88	3.29	41.83

Since the combustions of the material purified by the two methods and dried at the different temperatures are in excellent accord, it is evident that endothine red contains no water of crystallization.

Diacetylendothine red. Attempts to obtain the acetyl derivative of endothine red by the employment of anhydrous sodium acetate were unsuccessful. The material was, therefore, boiled with acetic anhydride for several hours, and toward the end the excess of reagent was removed by evaporation. On cooling a semicrystalline mass separated, which was filtered with suction, washed with absolute alcohol and ether, and recrystallized several times from warm absolute alcohol. A portion of the acetyl derivative is hydrolyzed to the original pigment when the alcohol contains an appreciable quantity of water. Even when preserved in a sealed tube for several months, darkening of the acetyl compound takes place. This change is rather remarkable when the pigment itself is so stable that cold concentrated nitric acid fails to attack it. The acetyl derivative was dried at 100° C. and combustions were made, with the results shown in table 2.

TABLE 2. *Combusion of Acetyl Derivative of Endothine Red*

Sample	Weight	CO_2	H_2O	C, Percent	H, Percent	O, Percent
A	.2000	.4034	.0720	55.01	4.03	40.97
B	.2007	.4047	.0723	54.99	4.03	40.98
C	.1576	.3217	.0553	55.60	3.93	40.47
Arithmetic mean of three determinations				55.20	4.00	40.80
Required for $C_7H_3O_4(C_2H_3O)_2$				55.67	3.82	40.51

The acetyl derivative forms glistening yellow lath-like crystals which probably belong to the triclinic system[8]. The compound melts with decomposition at about 184°–186° C. (uncorrected). Hawkins and Stevens give 196°–197° C. (uncorrected) as the melting point of a white acetyl derivative of endothine red. Repeated efforts to verify their statement[9] were not successful, since only the yellow derivative described above resulted in all trials.

The number of acetyl groups was ascertained by determining the acetic acid liberated, and the results were confirmed by determining the yield of endothine red recovered on hydrolysis of the diacetyl compound. Three methods were followed in estimating the acetic acid liberated on hydrolysis of the acetyl derivative.

The first method[10] consisted in boiling the compound in an Erlenmeyer flask for several hours with dilute barium hydroxide. The mixture was then acidified at ordinary temperature with phosphoric acid and the acetic acid distilled off. The distillate was treated with an excess of barium hydroxide, evaporated to a small volume and treated with carbon dioxide. The solution was then separated from the excess barium carbonate by filtration and the filtrate evaporated to dryness in a platinum dish. The residue was taken up in carbon dioxide-free water. The barium in solution was determined as sulphate.

For the second method[11] the acetyl derivative was placed in a flask with 30 cc. of alcohol and 2 cc. of sulphuric acid. The mixture was subjected to distillation until two thirds of the liquid had passed over. Fresh alcohol was then added and the operation repeated until the distillate gave no test for acetic acid. The distillate was collected in standard alcoholic potash which was subsequently heated a few minutes to hydrolyze the ethyl acetate formed and finally titrated with standard sulphuric acid.

In the third method[12] the substance was hydrolyzed with dilute sulphuric acid (75 parts of acid to 32 parts of water) on a hot water bath for one half hour, then diluted to 8 volumes and heated on a boiling water bath for 3 hours. The whole process was carried out under a reflux condenser. After 24 hours, the insoluble pigment was filtered off, washed with a little water, dried, and weighed. The filtrate was subjected to steam distillation until about 500 cc. had passed over. The distillate was titrated against standard alkali with phenolphthalein as an indicator. The results for the three methods are given in table 3.

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[8] The author is indebted to Mr. C. M. Smith, of the Insecticide and Fungicide Board, or the examination under the petrographic microscope of endothine red and its diacetyl derivative.

[9] In attempting to clarify this point, Dr. Hawkins assured the writer that the acetyl derivative described in their paper was obtained in small quantities and may have resulted from an impurity present with the pigment.

[10] Herzig, J. Studien über Quercetin und seine Derivate. I. Abhandlung. Monatshefte für Chemie **5**: 72–93. 1884.

[11] Perkin, Arthur George. The determination of acetyl groups. Journ. Chem. Soc. (London) Trans. **87**: 107–110. 1905.

[12] Liebermann, C. Ueber einige früher beschriebene Derivate des Quercetins. Ber. Deutsch. Chem. Ges. **17**: 1680–1684. 1884.

TABLE 3. *Determination of the Acetic Acid Liberated from Acetylendothine Red*

Method Used	Weight of Acetyl Comp.	$BaSO_4$	$C_2H_4O_2$	$C_2H_4O_2$, Percent
1st	.8986	.8122		46.45
2d	.3012		.1425	47.34
2d	.2723		.1275	46.80
3d	.2253		.1029	45.68
Arithmetic mean of four determinations				46.57
Required for				
$C_7H_4O_4(C_2H_3O)$				30.78
$C_7H_3O_4(C_2H_3O)_2$				50.64
$C_7H_2O_4(C_2H_3O)_3$				64.52

The percentage of endothine red in the acetyl compound was determined by weighing the pigment regenerated in the last of the three processes mentioned above for the determinations of the acetyl groups in the compound, as the pigment is only slightly soluble in dilute sulphuric acid. This percentage was also determined by another method[13] which consisted in hydrolyzing the diacetylendothine red in a small volume of acetic acid to which a little sulphuric acid was added. When the mixture was allowed to stand for a short time, red crystals began to separate out. By adding the mixture to several volumes of water and allowing it to stand twenty-four hours the separation was completed.

TABLE 4. *Percentage Yield of Recovered Endothine Red from Acetylendothine Red*

Method Used	Weight of Acetyl Compound	Recovered Pigment	Endothine Red, Percent
1st	.4532	.2938	64.82
2d	.1572	.1014	64.50
2d	.2253	.1457	64.66
Arithmetic mean of three determinations			64.66
Required for			
$C_7H_4O_4(C_2H_3O)$			78.97
$C_7H_3O_4(C_2H_3O)_2$			64.55
$C_7H_2O_4(C_2H_3O)_3$			54.48

The recovered endothine red was obtained as a "Brazil red" crystalline powder whereas the original coloring matter possessed a "ferruginous" color. On this account it was first thought that the recovered pigment had suffered a change in the process of hydrolysis. To decide this point the behavior of the two was studied more in detail. Both gave an "apricot orange" streak and acted in the same way toward such reagents as ferric chloride, dilute alkalis, sodium carbonate, and acids, and showed no differences when examined through the spectroscope. A spectral transmission curve of the pure endothine red is shown in figure 3, and, since the recovered pigment gave a similar curve, no doubt remains that the two compounds are identical.[14]

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[13] Perkin, Arthur G. Luteolin. Part I. Journ. Chem. Soc. (London) Trans. 69: 206–212. 1896.

Practically none of the violet rays are transmitted; in the blue and green portions of the spectrum there is slightly more transmission, while there is a gradually increasing transmission in the yellow, orange, and red portions.

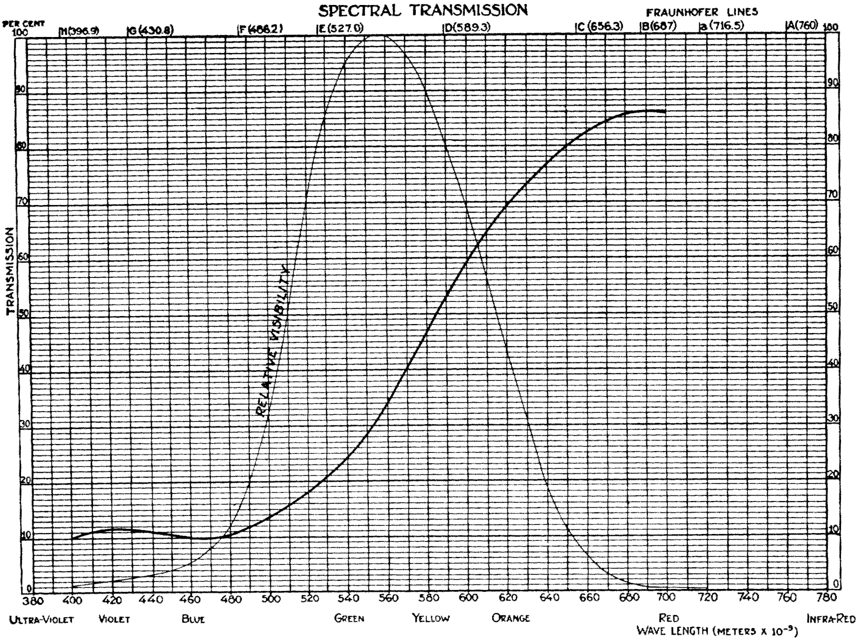

FIG. 3. Curve of percentage of spectral transmission of endothine red in alcoholic solution. Concentration, 0.00475 grams in 1 cc.

A combustion of the regenerated pigment furnished additional evidence of its identity with endothine red.

.1662 gm. gave .3340 gm. CO_2 and .0547 gm. H_2O.
Found C = 54.80, H = 3.68, O = 41.52.
Required for $C_7H_6O_4$ C = 54.88, H = 3.29, O = 40.83.

Molecular weight determination. Endothine red itself could not be used for the determination of its molecular weight on account of its slight solubility in solvents used for such determinations. It was found necessary, therefore, to use the diacetylendothine red for this purpose. The results with benzene as the solvent (K 2700) are reported in table 5.

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[14] The writer is indebted to Mr. I. C. Priest, of the Colorimeter Laboratory, Bureau of Standards, for this work.

TABLE 5. *Molecular Weight Determination of Diacetylendothine Red in Benzene*

Sample	Weight of Sample	Weight of Solvent	Elevation of the Boiling Point	Molec. Wt.
A	.0741	15.0351	.055°	241.9
B	.0848	"	.085	179.1
C	.0881	"	.065	243.4
D	.2470	"	.205	216.3
Arithmetic mean of four determinations				220.1
Required for $C_7H_3O_4(C_2H_3O)_2$				237.07

Disodium endothine red is formed on adding alcoholic sodium acetate to a boiling alcoholic solution of the coloring matter. It forms a "brownish olive" powder which is insoluble in alcohol, but readily soluble in water in which it gives a violet red solution. Apparently it undergoes no decomposition on boiling with water. The compound was dried at 100° and analyzed by heating in a muffle furnace for 6 hours.

.1600 gm. gave .0720 gm. Na_2CO_3. Found Na = 19.55.
.2106 gm. gave .0949 gm. Na_2CO_3. Found Na = 19.57.
$C_7H_3O_4Na_2.2H_2O$ requires Na = 19.77.

The water of crystallization was not determined. At the time of determination the possibility that the compound contained water of crystallization had not presented itself, and lack of material prevented further work on this point later.

Monobarium endothine red, $C_7H_3O_4Ba.2H_2O$, forms an olive-green felt-like mass when alcoholic barium acetate is added to a boiling alcoholic pigment solution. It is insoluble in cold water and in alcohol.

.2714 gm. gave .1638 gm. $BaCO_3$. Found Ba = 42.06.
.2823 gm. gave .2011 gm. $BaSO_4$. Found Ba = 41.92.
$C_7H_3O_4Ba.2H_2O$ requires Ba = 42.35.

THEORETICAL CONSIDERATIONS

In calculating the empirical formula for endothine red from the elementary analysis, three formulas seemed to give figures which are in fairly close agreement with the experimental values. *There seems to be no doubt that* $C_7H_5O_4$ *represents the true formula of this new coloring matter.* Table 6 gives the data from which the selection of the assigned formula was made.

The results of this investigation indicate that endothine red contains two hydroxyl groups and has acid properties which enable it to decompose alkali salts. Efforts to prepare an ester by prolonged digestion with alcohol and sulphuric acid in the usual manner failed. The acid properties can not, therefore, be ascribed to the presence of a carboxyl group. In this connection it is interesting to refer to the work of Perkin[15] which deals with

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[15] Perkin, Arthur George. A reaction of some phenolic coloring matters. Journ. Chem. Soc. (London) Trans. 75: 433–454. 1899.

TABLE 6. *Data Leading to the Selection of $C_7H_5O_4$ as the Formula of Endothine Red*

Found		Required for					
		$C_7H_5O_4$		$C_9H_7O_5$		$C_{14}H_{11}O_8$	
C	H	C	H	C	H	C	H
55.00	3.53	54.88	3.29	55.27	3.61	54.77	3.61
54.62	3.58						
54.40	3.58						
54.87	3.51						
mean							
54.73	3.55						
Acetyl Derivative		$C_7H_4O_4(C_2H_3O)$ = 55.27 C 3.61 H		$C_9H_6O_5(C_2H_3O)$ = 55.67 C 3.82 H		$C_{14}H_8O_8(C_2H_3O)_3$ = 55.40 C 3.96 H	
C	H						
55.01	4.03	$\boxed{C_7H_3O_4(C_2H_3O)_2 = 55.67\ C\quad 3.82\ H}$		$C_9H_5O_5(C_2H_3O)_2$ = 55.89 C 3.97 H		$C_{14}H_7O_8(C_2H_3O)_4$ = 55.56 C 4.03 H	
54.99	4.03						
55.60	3.93	$C_7H_2O_4(C_2H_3O)_3$ = 55.89 C 3.97 H		$C_9H_4O_5(C_2H_3O)_3$ = 56.05 C 4.08 H		$C_{14}H_6O_8(C_2H_3O)_5$ = 55.68 C 4.09 H	
mean							
55.20	4.00						
Yield of Regenerated Pigment 64.82		$C_7H_4O_4(C_2H_3O)$ = 78.97		$C_9H_6O_5(C_2H_3O)$ = 82.27		$C_{14}H_8O_8(C_2H_3O)_3$ = 70.08	
64.50 64.66		$\boxed{C_7H_3O_4(C_2H_3O)_2 = 64.55}$		$C_9H_5O_5(C_2H_3O)_2$ = 69.89		$C_{14}H_7O_8(C_2H_3O)_4$ = 64.62	
mean 64.66		$C_7H_2O_4(C_2H_3O)_3$ = 54.48		$C_9H_4O_5(C_2H_3O)_3$ = 60.74		$C_{14}H_6O_8(C_2H_3O)_5$ = 59.37	
Acetic acid from Acetyl Derivative 46.45		$C_7H_4O_4(C_2H_3O)$ = 30.78		$C_9H_6O_5(C_2H_3O)$ = 25.32		$C_{14}H_8O_8(C_2H_3O)_3$ = 41.81	
47.34 46.80		$\boxed{C_7H_3O_4(C_2H_3O)_2 = 50.64}$		$C_9H_5O_5(C_2H_3O)_2$ = 43.01		$C_{14}H_7O_8(C_2H_3O)_4$ = 50.53	
45.68 mean 46.57		$C_7H_2O_4(C_2H_3O)_3$ = 64.52		$C_9H_4O_5(C_2H_3O)_3$ = 56.08		$C_{14}H_6O_8(C_2H_3O)_5$ = 58.03	
Regenerated Pigment		C	H	C	H	C	H
C	H						
54.80	3.68	54.88	3.29	55.27	3.61	55.77	3.61
Molecular weight of Acetyl Compound 179.1 (?)		$C_7H_4O_4(C_2H_3O)$ = 195.06		$C_9H_6O_5(C_2H_3O)$ = 237.07		$C_{14}H_8O_8(C_2H_3O)_3$ = 433.14	
241.9 216.3		$\boxed{C_7H_3O_4(C_2H_3O)_2 = 237.07}$		$C_9H_5O_5(C_2H_3O)_2$ = 279.09		$C_{14}H_7O_8(C_2H_3O)_4$ = 475.15	
243.4 mean 220.1		$C_7H_2O_4(C_2H_3O)_3$ = 279.09		$C_9H_4O_5(C_2H_3O)_3$ = 321.10		$C_{14}H_6O_8(C_2H_3O)_5$ = 517.17	
Na from Sodium Derivative		$C_7H_4O_4Na$ = 13.16 $C_7H_3O_4Na_2$ = 23.38		$C_9H_6O_5Na$ = 10.59 $C_9H_5O_5Na_2$ = 19.27		$C_{14}H_9O_8Na_2$ = 13.13 $C_{14}H_8O_8Na_3$ = 18.52	
19.55 19.57		$\boxed{C_7H_3O_4Na_2, 2H_2O = 19.77}$					
mean 19.56		$C_7H_2O_4Na_3$ = 31.54		$C_9H_4O_5Na_3$ = 26.47		$C_{14}H_7O_8Na_4$ = 23.32	
Ba from Barium		$C_7H_3O_4Ba$ = 47.63		$C_9H_5O_5Ba$ = 41.57		$C_{14}H_7O_8Ba_2$ = 47.54	
Derivative 42.06		$\boxed{C_7H_3O_4Ba, 2H_2O = 42.35}$					
41.92 mean 41.98							

the acid properties of coloring matters of the anthraquinone, flavone, xanthone, and ketone groups. He says: "With the exception of morin and rhamnazin, no coloring matter of known constitution is markedly acidic unless it contains two hydroxyls in the ortho-position relatively to one another, and consequently has strong dyeing properties." Further evidence that the two hydroxyls are in the ortho-position in endothine red was obtained by fusing the compound with potash. By heating the pigment to 200°–250° C. for half an hour with ten times its weight of potassium hydroxide, dissolved in a little water, a phenol portion was obtained, which gave a green coloration with ferric chloride. The fusion was carried out several times, and at no time could the phenol be isolated in sufficient quantities for further identification. It is probable, however, that it is pyrocatechin or a member of this group since this class of phenols gives a green color with ferric chloride. A red acid portion of the potash fusion gave a greenish "raw sienna" color with ferric chloride and appeared to consist partly at least of the unchanged pigment.

The foregoing considerations justify the conclusion that endothine red is probably related to the members of the pyrocatechin group. The exact constitution of a side chain, if such exists, and further confirmation of the meager evidence at hand will, it is hoped, be the subject of another investigation.

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